Hiroshi OKADA* & Michio TAMURA*: **Karyomorphology and relationship on the Ranunculaceae****

岡田 博*・田村道夫*: キンポウゲ科の核形態と類縁関係**

Numerous karyological contributions have been made toward the phylogenetic classification of the Ranunculaceae. Langlet (1932) divided the Ranunculaceae, excluding *Paeonia*, *Glaucidium* and *Hydrastis*, into two subfamilies mainly based on the chromosome size and curvature, *i.e.*, one was of "lange, im allgemeinen gewundene oder mehrfach gebogene, meist recht grosse" chromosomes, namely, R-type and another was of "im allgemeinen einfach gebogene, verhältnismässig kleine" ones, namely, T-type. Gregory (1941) followed Langlet and approved of the distinction of R- and T-type in this family including *Paeonia*. However, he intended to attach more importance to the basic chromosome number and to recognize C-type in *Coptis* and *Xanthorhiza*. As the result of these karyological and other morphological evidences, Tamura (1962, 1967) concluded that the karyological feature is the most important character for the classification of this family. He divided this family excluding *Paeonia* and *Glaucidium* into six subfamilies. Rothfels *et al.* (1966) showed that DNA values within the Ranunculaceae were closely correlated with total length of the chromosome complement, so that the variations in chromosome size corresponded directly to the variations in DNA values. They suggested that the chromosome size might be reliable as the character by which this family was divided. On the other hand, however, total sum of chromosome length in *Allium cepa* varied ranging from 98 μ to 242 μ among the cells without pretreatment, and from 149 μ to 195 μ among the cells with colchicine pretreatment (Tanaka & Tanaka 1975). In *Vicia faba*, while DNA content remained constant, nuclear RNA and histone ratio varied significantly among the cells of different ages and tissues, and the chromosome volume is changeable between 274 μ^3 and 638 μ^3 (Bennett 1970).

* Department of Biology, College of General Education, Osaka University, Toyonaka, Osaka. 大阪大学教養部生物学教室

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Accordingly, for the comparison of chromosome size of diffferent species the method of pretreatment, the kind of meristem and the stage in cell division should be regulated strictly.

In the previous cytotaxonomical studies the phylogeny and relationships of the taxa were discussed on the basis of number, size and form of mitotic metaphase chromosomes. Besides these characters, however, the distribution pattern of euchromatin and heterochromatin at interphase and prophase seems to be very important. The previous observations show that the distribution pattern of both chromatins is stable within the taxa.

As a result of observation of the nuclei at interphase and prophase in the Ranunculaceae including *Paeonia*, Kurita (1960) pointed out that nuclei of T-type and of R-type showed the different distribution pattern of darkly stained regions from each other. There are many evidences that the distribution patterns of both chromatins are well coincident with natural groups as in Onagraceae (Kurabayashi *et al.* 1962), in Orchidaceae (Tanaka 1971), in woody Polycarpicae (Okada 1975, Okada & Tanaka 1975), and so on.

In this paper, we report the distribution patterns of both chromatins at mitotic interphase and prophase in detail and the chromosome size at metaphase observed on 33 species belonging to 16 genera of the Ranunculaceae including *Paeonia* and *Glaucidium*, and discuss the relationship of this family based on the results.

Materials and methods Materials and their sources investigated are shown in Table 1. Preparations for observation of mitotic phases were carried out as follows: the root tips were pretreated with 0.002 M 8-hydroxyquinoline aqueous solution for four to six hours at room temperature, and fixed in the 2: 1: 1 mixture of absolute ethanol, chloroform and glacial acetic acid at 5°C for over one hour. These root tips were macerated in 1N HCl for ten to fifteen seconds at 60°C, and stained with 1% aceto-orcein for 20 to 30 minutes. They were squashed with the conventional techniques.

Observations were made on nuclei at the mitotic interphase, prophase and metaphase. The two daughter cells before enlargement of cell size were used in observation of interphase nuclei, because the nuclei vary slightly their distribution patterns of chromatins during interphase, so that, except this stage, it was difficult to identify the corresponding stage on different species. We confirmed preliminarily that the distribution pattern of eu- and

Table 1. Sources, chromosome numbers and chromosome length of investigated species.

Species	Source	2n	Length (μ) max.-min.
Paeonia japonica	Mt. Tanzawa, Kanagawa Pref.	10	15-10
P. lutea var. *ludlowii.*	EBG*	10	13-10
Cimicifuga japonica	Mt. Kongo, Osaka Pref.	16	10- 5
Nigella damascena	cult.	12	12- 5
Aconitum sanyoense	Ashiu, Kyoto Pref.	16	10- 2
A. grosse-dentatum	Mt. Kongo, Osaka Pref.	32	10- 2
A. triphyllum	Izumi-Sunagawa, Osaka Pref.	32	10- 2
A. ibukiense	Aogaki, Hyogo Pref.	32	10- 2
A. japonicum var. *eizanense*	Ohara, Kyoto Pref.	32	10- 2
A. yezoense	Jozankei, Hokkaido Pref.	32	10- 2
Anemone nikoensis	Mt. Kongo, Osaka Pref.	14	12- 8
Hepatica insularis	Isl. Cheju-do Korea.	14	12- 8
Knowltonia bracteata	EBG*	48	8- 4
Clematis stans	Hakone, Kanagawa Pref.	16	10- 5
C. lasiandra	Mt. Kôya, Wakayama Pref.	16	9- 4
C. patens	Sanda, Hyogo Pref.	16	10- 5
C. meyeniana	Isl. Okinawa, Okinawa Pref.	16	10- 5
Ranunculus japonicus	Mt. Kongo, Osaka Pref.	14	8- 4
R. siamensis	Mae-Sanam, Thailand.	16	8- 4
R. silerifolius	Minoo, Osaka Pref.	16	7- 4
R. cantoniensis	Minoo, Osaka Pref.	32	7- 4
R. sceleratus	Minoo, Osaka Pref.	32	3- 1.5
R. sieboldii	Isl. Yaku, Kagoshima Pref.	48	6- 3
R. nipponicus var. *major*	Hakone, Kanagawa Pref.	48	3- 1
Dichocarpum stoloniferum	Mt. Tanzawa, Kanagawa Pref.	35	2- 1.5
Aquilegia flabellata	cult.	14	2- 1.5
Thalictrum filamentosum	Mt. Ôdai, Nara Pref.	14	1.5- 1
T. minus var. *hypoleucum*	Sasayama, Hyogo Pref.	42	1.5- 1
Coptis japonica	Mt. Kôya, Wakayama Pref.	18	2.5- 2
C. ramosa	Isl. Yaku, Kagoshima Pref.	18	2- 1.5
Xanthorhiza apifolia	NBG**	36	1- 0.5
Hydrastis canadensis	NBG**	26	2- 1
Glaucidium palmatum	Mt. Gassan, Yamagata Pref.	20	2.5- 1.5

* EBG: Royal Botanic Garden, Edinburgh. ** NBG: Nikko Botanical Garden, University of Tokyo.

heterochromatin does not change during prophase, and used mid-prophase for the description of prophase chromosomes. During prophase and metaphase the chromosomes become shorter. The shortening is finished when they arrange themselves at the equatorial plate. By the treatment of 8-hydroxyquinoline the process of nuclear division is inhibited at metaphase, and variation in chromosome size becomes to reduce. But the preparations still include a few cells which are in prometaphase. For taking up the metaphase, we selected the shortest chromosome set among several cells observed and measured the length of the largest chromosome and the smallest one.

Observations Results of chromosome counts and length of the largest and the smallest chromosome at metaphase are shown in Table 1.

Among the species observed three types of distribution pattern of chromatins at interphase and prophase were distinguished.

First type. In the interphase nuclei no condensed body is observed. Chromatin is stained as small granules and spreaded over the nucleus uniformly (Fig. 1A). The chromosomes at prophase look like homogeneous long threads. Eu- and heterochromatic segments are not distinguishable from each other (Fig. 1B). The species belonging to this type are *Paeonia japonica* (Fig. 1) and *P. lutea* var. *ludlowii*.

Second type. In the interphase nuclei a few small condensed bodies stained darkly are observed. The other region is stained unevenly and granulous structure does not appear (Figs. 2A, 3A, 4A). In the prophase chromosomes eu- and heterochromatic segments are distinguishable, but boundaries of both segments are indistinct, and the transition is gradual. The heterochromatic segments are distributed in proximal, distal or interstitial regions of both arms, and much shorter than euchromatic segments (Figs. 2B, 3B, 4B). The species belonging to this type are as follows: *Cimicifuga japonica*, *Nigella damascena*, *Aconitum sanyoense*, *A. grosse-dentatum*, *A. triphyllum*, *A. ibukiense*, *A. japonicum* var. *eizanense*, *Anemone nikoensis*, *Hepatica insularis* (Fig. 2), *Knowltonia bracteata* (Fig. 3), *Clematis stans*, *C. lasiandra*, *C. patens*, *C. meyeniana*, *Ranunculus japonicus*, *R. siamensis*, *R. silerifolius* (=*R. quelpaertensis*), *R. cantoniensis*, *R. sceleratus*, *R. sieboldii* and *R. nipponicus* var. *major* (Fig. 4).

In many species, values of chromosome length fall into the range from 10μ to 2μ usually (Table 1), but in *R. sceleratus* (3-1.5μ) and *R. nipponicus*

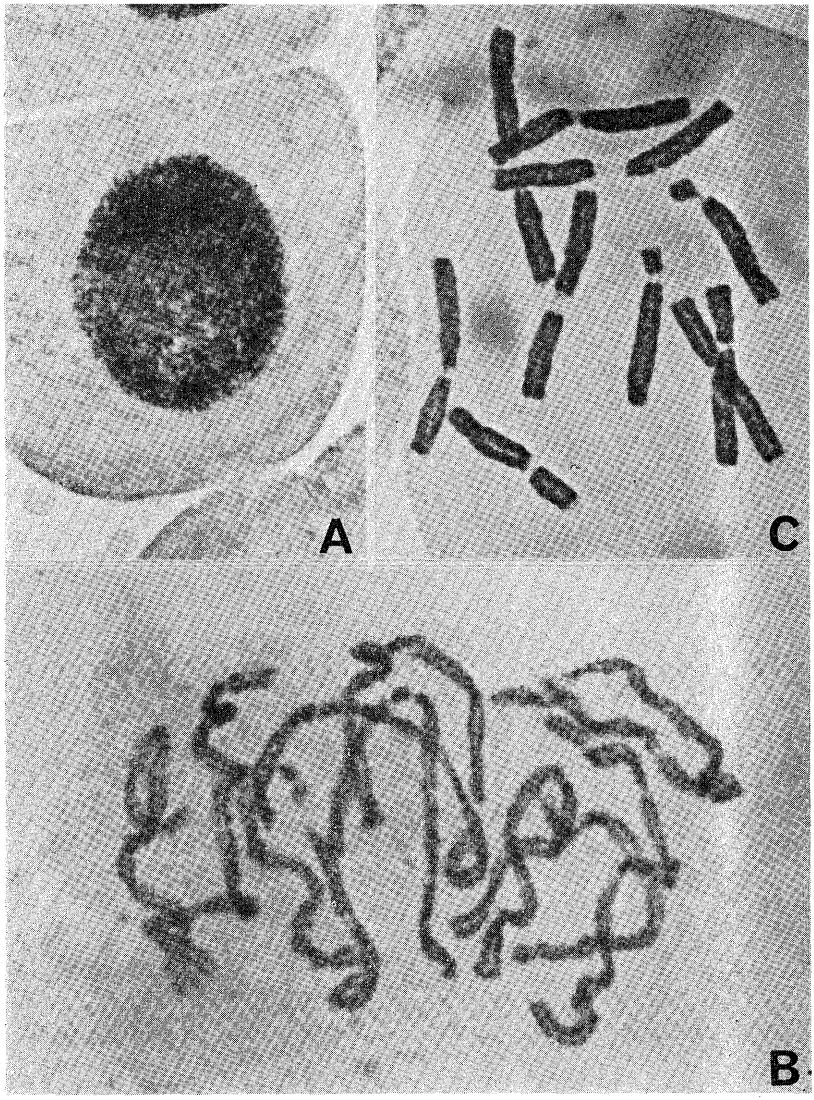

Fig. 1. Somatic chromosomes of *Paeonia japonica*. 2n=10. A. Interphase. B. Prophase. C. Metaphase. ×1800.

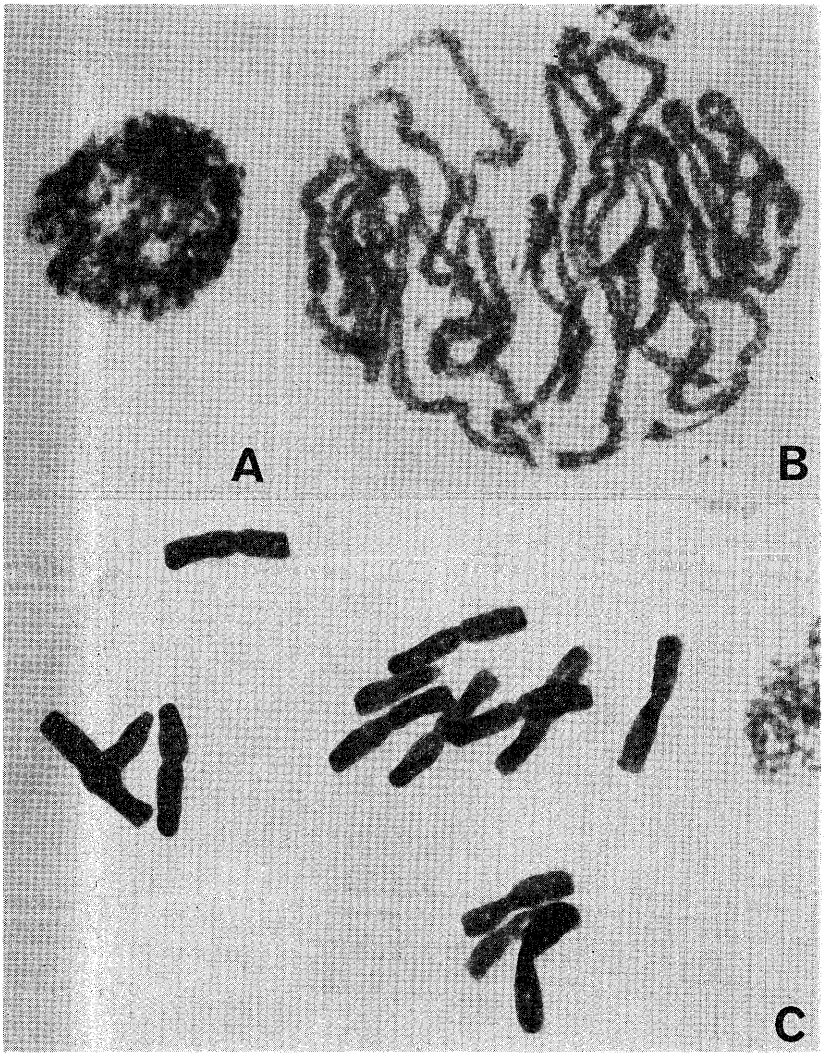

Fig. 2. Somatic chromosomes of *Hepatica insularis*. 2n=14. A. Interphase. B. Prophase. C. Metaphase. ×1800.

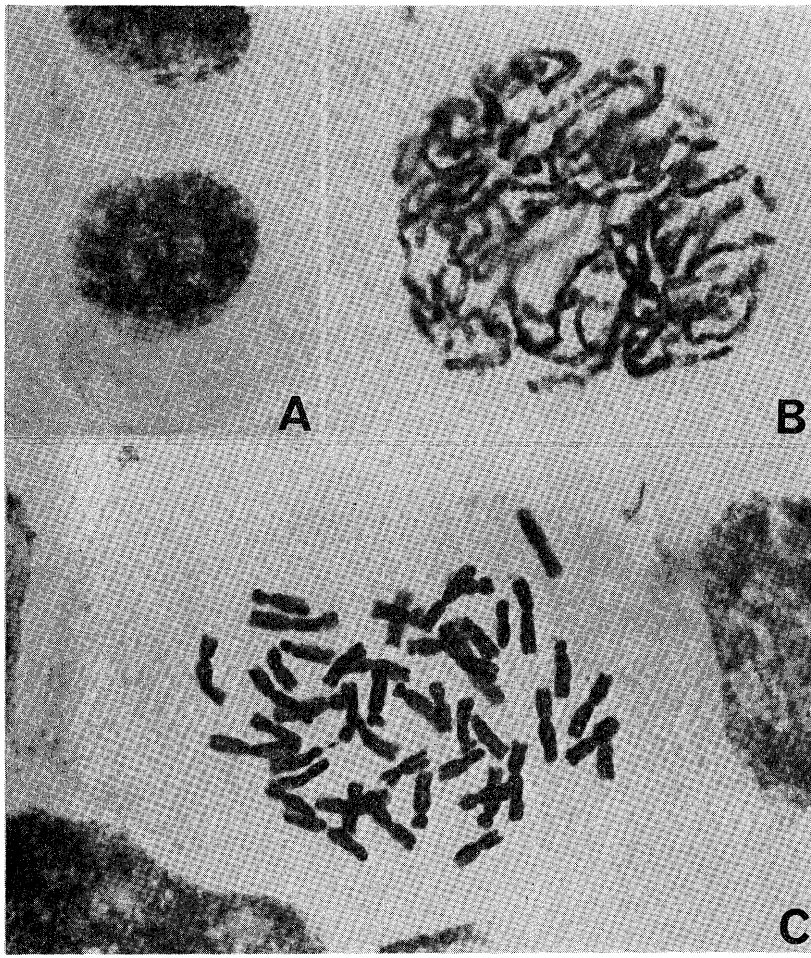

Fig. ?. Somatic chromosomes of *Knowltonia bracteata*. 2n=48. A. Interphase. B. Prophase. C. Metaphase. ×1800.

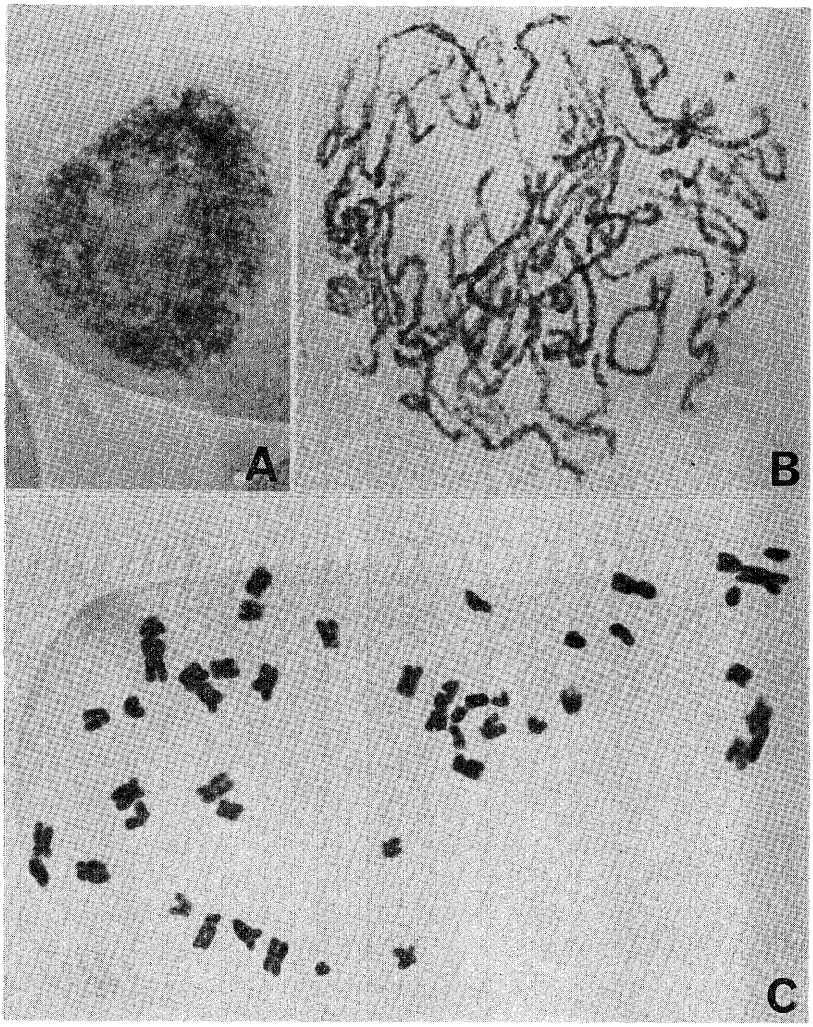

Fig. 4. Somatic chromosomes of *Ranunculus nipponicus* var. *major*. 2n=48. A. Interphase. B Prophase. C. Metaphase. ×1800.

var. *major* (3-1μ) the chromosomes are especially small (Fig. 4C), on the contrary, in *N. damascena* (12-5μ), *A. nikoensis* (12-9μ) and *H. insularis* (12-8μ) they are especially long (Fig. 2C).

Third type. In the interphase nuclei many condensed bodies stained darkly are observed. Their relative size for nucleus is larger than those of the former type. Chromatin surrounding the condensed bodies is diffused, and the boundary of the bodies is slightly indistinct. The other region is stained evenly and more dilutely than in the first and the second types, and granulous structure does not appear (Figs. 5A, 6A). At prophase chromosomes both eu- and heterochromatic segments are well-differenciated and the boundary is distinct. The heterochromatic segments are distributed in proximal region of both arms (Figs. 5B, 6B), and slightly shorter than euchromatic segments. The species belonging to this type are: *Dichocarpum stoloniferum*, *Aquilegia flabellata*, *Thalictrum filamentosum*, *T. minus* var. *hypoleucum*, *Coptis japonica*, *C. ramosa* (Fig. 5), *Xanthorhiza apiifolia*, *Hydrastis canadensis* and *Glaucidium palmatum* (Fig. 6).

Among these species, however, *Coptis japonica* and *C. ramosa* (Fig. 5B) are slightly different karyomorphologically from the other. That is, the boundary between both chromatic segments at prophase is not so distinct as in the other, *e.g.*, *G. palmatum* (Fig. 6B), and euchromatic segments are considerably shorter than heterochromatic segments. In *X. apiifolia* each of

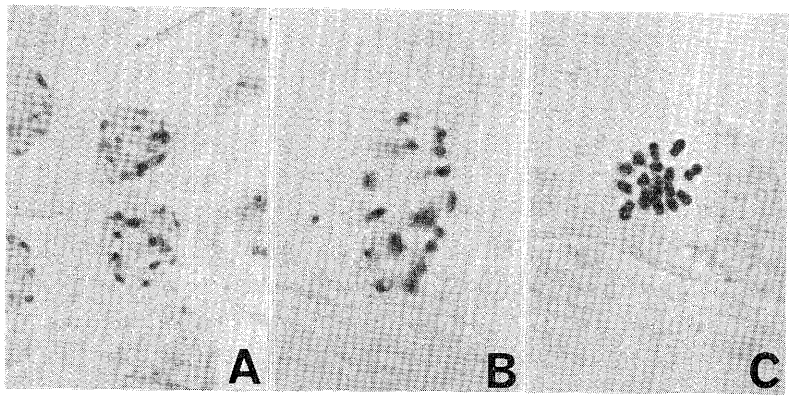

Fig. 5. Somatic chromosomes of *Coptis ramosa*. 2n=18. A. Interphase. B. Prophase. C. Metaphase. ×1800.

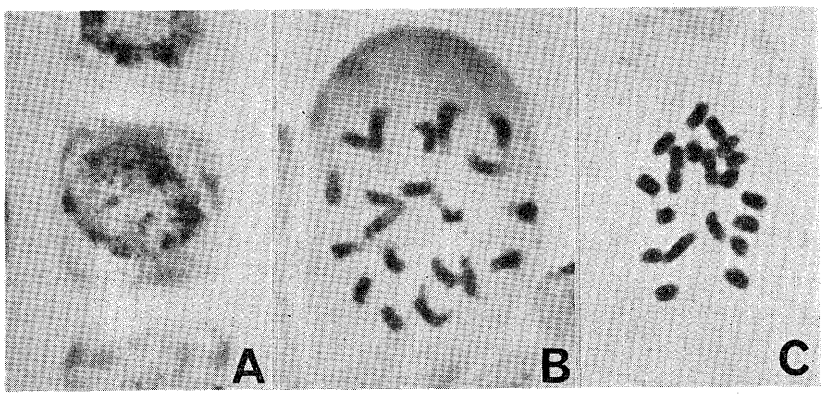

Fig. 6. Somatic chromosomes of *Glaucidium palmatum*. 2n=20. A. Interphase. B. Prophase. C. Metaphase. ×1800.

the largest chromosome and the smallest one shows minimum value in length among the species investigated (Table 1).

Discussion In this investigation we classified 33 species of 16 genera into three groups on the basis of karyomorphology. According to Kurita (1960) the species classified here in the first and the second group have R-type chromosomes and those in the third group have T-type ones, and *Paeonia* was classified in R-type. However, in our observations two species of *Paeonia* were clearly distinguishable from other species as described above. This evidence supports the opinion that *Paeonia* has little relation with the Ranunculaceae.

The species belonging to the second group correspond to those with R-type chromosomes, but the length of metaphase chromosomes shows considerable difference among species as mentioned above, *e.g.*, *H. insularis* 12-8 μ (Fig. 2C), *Knowltonia bracteata* 8-4μ (Fig. 3C) and *R. nipponicus* var. *major* 3-1μ (Fig. 4C). *R. nipponicus* var. *major* and *R. sceleratus* possess the chromosomes only about a half in length, comparing with other species of *Ranunculus* (8-3μ). Considering together with other characters these small chromosomes may be originated secondary reduction through the process of speciation.

The basic chromosome numbers of this group are found to be x=6, 7 and 8. Among these numbers, x=8 is considered as the original basic number for this group judging from the combination with other characters, and x=7

and 6 may be derived by the aneuploidal reduction as already stated (Tamura 1966, 1967), though Gregory thought in *Nigella* x=7 was derived from x=6, not from x=8.

The karyomorphology of the species included in the third group were found to correspond to T-type, similar to the results of Kurita (1960). In the case of *C. japonica* and *C. ramosa*, the transition from eu- to heterochromatic segments at prophase chromosomes is not so distinct, and the length of euchromatic segments are shorter considerably than those in the other species. So the chromosomes of *Coptis* seem to get slightly near the second type. While, *X. apiifolia* has very small chromosomes of about a half of those of *Coptis* in length. Accordingly, there is little reason to distinguish the chromosomes of *Coptis* and *Xanthorhiza* from those of other genera as C-type as Gregory (1941) intended.

In the third group there are four basic chromosome numbers, *i.e.*, x=7, 9, 10 and 13. Except x=10 of *Glaucidium*, species having each basic chromosome number is classified into Isopyroideae-Thalictroideae, Coptidoideae and Hydrastidoideae, respectively. These numerical variations among subfamilies show a discontinuous line in contrast to the aneuploid series in the second group, Helleboroideae-Ranunculoideae. This fact suggests that, even if they have a common ancestral group, the differentiation in this group took place much earlier than in the second group.

Basic chromosome number x=10 of *Glaucidium* may be considered to show the relation to x=5 in *Paeonia*, however, the distribution pattern of both chromatins shows quite different features (Figs. 1, 7). On the basis of karyomorphology it is very difficult to prove the close relationship between both genera. *Glaucidium* is regarded as the representative of the Glaucidiaceae, separated from the Ranunculaceae, but its karyomorphology is not different from the third type.

Summary Karyomorphological observations were carried out in 33 species of 16 genera belonging to the Ranunculaceae (including *Paeonia* and *Glaucidium*), and discussed the relationship of these species based on the observations.

Three karyomorphological types were recognized. The second type and the third type correspond to R-type and T-type which have been proposed by Langlet (1932), respectively. The second type is observed in the species

belonging to Ranunculoideae and Helleboroideae, and the third type is observed in Isopyroideae, Thalictroideae, Coptidoideae and Hydrastidoideae.

The distribution patterns of *R. sceleratus* and *R. nipponicus* var. *major* show distinctly of the second type, though their chromosomes are exceptionally small.

The first type is observed only in *Paeonia*. This evidence supports the opinion that *Paeonia* has little relationship with the Ranunculaceae.

Basic chromosome number x=10 of *Glaucidium* might be considered to relate to x=5 of *Paeonia*, but on the basis of karyomorphology it is very difficult to prove the close relationship between both genera.

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キンポウゲ科の16属33種（ボタン属とシラネアオイ属を含む）の植物の核形態を観察し，これらの植物の類縁関係を考察した。

3型の核形態が識別された。

第1の型：中間期核の染色質は顆粒状に，均等に染まり，凝縮塊は見られない。前期染色体は均質な糸状をしていて，真正染色質，異質染色質は見分けられない。

第2の型：中間期核には2-数個の小さな凝縮塊が見られる。その他の部分は不均等に染まり，また顆粒状を呈しない。前期染色体では両染色質は区別できるが，その境界は不明瞭である。異質染色質は染色体腕の基部，端部，介在部に分布する。

第3の型：中間期核には数個の，前型に比べ相対的にやや大きい凝縮塊が見られる。それらの周辺部はやや分散状で，他の部分との境界がやや不明瞭である。凝縮塊以外の部分はほぼ均等に，また前型に比べうすく染まる。前期染色体では両染色質は明瞭に区別される。異質染色質は染色体腕の基部に分布する。

第2の型は Langlet (1932) の R 型，すなわち大形の染色体に，第3の型は T 型，すなわち小形の染色体に対応する。第2の型はキンポウゲ亜科，クリスマスローズ亜科の植物で見られ，第3の型はシロカネソウ亜科，カラマツソウ亜科，オウレン亜科，ヒドラスチス亜科の植物で見られた。小さな染色体を持つタガラシとバイカモでは両染色質の分布パターンは同属の他の種と同じく第2の型を示した。

第1の型はボタン属のみに見られた。この結果はボタン属が他のキンポウゲ科植物と類縁がうすいという意見を支持するものである。

シラネアオイ属の基本数 x=10 はボタン属の x=5 と関連があるように思えるが，核形態の上からは両属は関連は認められない。

□初島住彦・天野鉄夫：**琉球植物目録** Flora of the Ryukyu. 230頁. 1977年10月. でいこ出版社。1958年に出版された「沖縄植物目録」と全く同じ体裁であり，その改訂版であるが，新版では奄美群島まで収録範囲が広げられ，したがって書名も琉球と変えられている。前著からはずいぶん学名が変っているし，初島氏の「琉球植物誌」1971年と較べてもかなり学名の変更があり，新しく琉球に分布することのわかったものも目につく。琉球植物の解明には東南アジアの植物との比較研究は欠かせないもので，まだまだ長い年月と地道な研究が必要なことを感じる。（山崎 敬）